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Management of Varroa Mites in Honey Bees

By R.W. Currie
Dept. of Entomology,
University of Manitoba,
Winnipeg, Manitoba, Canada
R3T 2N2

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Summary:

Winter Apistan treatments did not have a negative impact on colony survival or spring brood production either in colonies that were treated in winter only and compared to those left untreated or in those colonies that were treated in both fall and winter and compared to those treated only in the fall. Our two year study showed that when both tracheal and varroa mites were present, the practice of leaving Apistan strips in colonies resulted in a trend towards larger colonies and greater colony survival in one year and significantly larger colonies and a trend towards greater colony survival in the second year.

Bees did not have constant contact with the strip throughout the winter, however, the amount of time that bees did spend in contact with the strip (84 to 98 days) exceeds that found on the current label recommendation. When fluvalinate strips were left inside colonies throughout the winter the resulting residue level in the comb ranged from 0.005 to .135 ppm (mean 0.04 ± 0.014, indoors; 0.0025 ±0.0025ppm, outdoors). Fluvalinate residues in indoor-wintered colonies tended to be higher than in outdoor wintered colonies however these differences were not significant. Further analysis of comb residues will give important information on the potential impact of this practice on the development of pesticide resistance in mites. This data should be carefully considered in determining wether a change in the pesticide label recommendation is warranted.

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Management of Varroa Mites in Honey Bees

Technical Report

Background and Objectives

The varroa mite is an external parasite of the honey bee that causes considerable economic damage. To date, there are only two chemicals registered for controlling this parasite, fluvalinate strips and formic acid. Fluvalinate strips have proven to be an effective and economical control measure for this parasite, however, they must be removed from colonies during the honey flow. Current label recommendations also indicate that they must not be left in colonies for periods greater than 42 days and must be disposed of after only one use. The first recommendation was established to prevent the potential for buildup of fluvalinate residues in wax or honey, and to reduce the length of time when selection pressure was applied to the mites in hopes of delaying the development of pesticide resistance. The second recommendation was designed to avoid exposing the mites to sublethal doses of fluvalinate, again, in hopes of delaying development of resistance.

Removal of the strips is undesirable because in late-fall this involves opening colonies and disturbing them. In some cases colonies that have been prepared for winter must be unwrapped and lids that have been previously sealed with propolis by the bees cannot be resealed because temperatures are too cool. There is a desire on the part of many beekeepers to be able to treat colonies in the fall using apistan strips and to leave strips in colonies throughout the winter period. The effects this may have on the release-rate of fluvalinate from the strips in colder temperatures, the potential efficacy of the strips under these conditions, and the potential for residue build up in the comb are not known. In order to determine if leaving strips in colonies may be a viable management technique that could be added to the label, the performance of strips in this situation, the potential length of selection pressure, the potential for residues in wax and honey, and the potential negative impacts on colony performance must be assessed.

The objectives of this research are: 1. to determine the release of fluvalinate from Apistan strips if they are left in over wintered colonies on the prairies; 2. to assess the impact of this application method on residues in honey and wax; 3. to determine the efficacy of treatment and estimate the potential selection pressures on mites; and 4. to estimate the impact of long-term exposure on colony performance.

Materials and Methods

In the fall of 1998, thirty two colonies of honey bees were randomly assigned to each of the following four treatments: 1. Winter treatment with Apistan with colonies wintered outdoors; 2. Winter treatment with Apistan with colonies wintered indoors; No apistan treatment with colonies wintered outdoors; 4. No Apistan treatment with colonies wintered indoors. None of the hives used in this experiment had received previous treatment with Apistan.

All of the colonies were housed in double chamber standard Lanstroth hives. Colonies that were wintered outdoors were provided with an inner cover having a top entrance. These hives were then covered by a layer of R-12 fiberglass insulation and wrapped with black plastic. Indoor-wintered colonies also were provided with an inner cover having a top entrance but were not wrapped. Indoor-wintered colonies were maintained at 5^0C in constant darkness in the University of Manitoba honey bee wintering building.

Prior to treatment with Apistan a sample of approximately 250 worker bees was collected from each hive. These samples were analysed to determine the incidence of *Varroa destructor* (Anderson) and *Acarapis woodii* and to assess the *Nosema apis* infestation level in each hive. Colonies were sampled by brushing about 250 adult bees from the middle frame of the top brood chamber into a sample bottle. In the laboratory, the bees were put into a basket made of plywood 21 x 21 cm wide x 7 cm deep with an eight mesh wire bottom. This was placed in a closed plastic container measuring 29 x 20 x 8 cm containing enough 70% ethanol to cover the bees. The sample was agitated for five minutes using a Lab-line orbital shaker rotating at 150 rpm. This sampling method was determined to be effective by shaking bees for an additional 30 minutes after the initial five minutes and examining the sample and each bee for additional mites. No mites were found on either adult bees nor in the alcohol from samples shaken the second time.

After samples were shaken, the basket with bees was removed, the mites in the alcohol were counted, and a mite/bee ratio was determined. A subsample of 100 of these bees was taken to determine the relative incidence of tracheal mites and the infestation level of *Nosema apis*. The incidence of tracheal mites was determined by counting the number of bees with at least one infected trachea. *Nosema* infestation level was assessed by determining the average number of spores per bee. On 17 May 1999, a second sample of bees was collected from colonies that survived the winter to determine the varroa mite infestation level.

Apistan treatments were applied on November 9th, 1998. Two Apistan® strips were hung in the upper brood chamber for the entire winter, one between frames three and four and one between frames seven and eight.

On April 29th 1999, a single comb was selected from the centre of each brood chamber of both treated and control colonies and stored for subsequent residue analysis.

A second experiment was conducted in the fall of 1999 using a total of thirty two colonies

of honey bees were obtained from two commercial beekeepers. Each beekeeper's colonies were randomly assigned to each of the following four treatment groups: 1. Fall treatment using Apistan with colonies wintered outdoors; 2. Fall and Winter treatment using Apistan with colonies wintered indoors; Fall treatment using Apistan with colonies wintered outdoors; and 4. Fall and Winter treatment using Apistan with colonies wintered indoors.

All colonies from beekeeper one were treated with Apistan on the 16th of September and also received a single treatment of formic acid at that time. Approximately 40 ml of formic acid was applied using a mite wipe® pad. Beekeeper two treated all colonies on the October 1st but did not use any formic acid treatment. In each case strips were removed from colonies 42 days after treatment. Wintering procedures were as described for 1998, above. On October 1st Hobo® data loggers were inserted into each hive. The logger contained an internal temperature and humidity probe and was affixed to one of the strips. The loggers external temperature probe was affixed to the other strip. The hobos were used to monitor temperature and relative humidity adjacent to the strip at 15 minute intervals throughout the winter months. The amount of contact that bees had with the strips was estimated by comparing the temperature next to the strip with ambient temperatures in control colonies with no bees.

On 27 March, 2000 each colony was opened and the cluster size was ranked according to the number of frames of bees present. On April 27th each colony was assessed to determine the worker population and sealed brood area.

Colonies were sampled for Varroa, Tracheal mite and nosema on 1 October, 1999 and on April 27th, 2000.

On 27th April 2000, a single comb was selected from the centre of each brood chamber of both treated and control colonies and stored for subsequent residue analysis.

Residue analyses were carried out according to standard methodology as outlined in Frison *et al.* (1999).

Results

Colonies that received a winter treatment with Apistan in 1998-1999 had higher pretreatment levels of both varroa and tracheal mites when compared to untreated colonies (Table 1). Winter treatment with Apistan reduced the abundance of varroa mites in indoor wintered colonies by over 72% (Table 1). Comparision of the pretreatment level of mites in colonies that died and survived over the course of the winter showed that colonies that had been treated with Apistan were able to tolerate higher pre-treatment populations of varroa and tracheal mites than colonies that received no apistan treatment (Table 1).

Colony populations were not significantly reduced by winter treatments with Apistan (Figure 1). In contrast both indoor and outdoor wintered colonies showed a trend to higher bee populations relative to untreated colonies when the strips were left in colonies during the winter

months. Outdoor colonies also had more colonies surviving in the treatment that had strips left in during the winter than in the control group (Figure 1). However, this difference in colony survival rate was not significant. Spring brood production at the time of sampling did not differ with either the method of wintering or the application of acaricide treatment(Figure 2). In these colonies where both varroa and tracheal mites post-winter colony populations were significantly higher in indoor-wintered colonies than in outdoor wintered colonies.

When fluvalinate strips were left inside colonies throughout the winter the residue level in comb ranged from 0.005 to .135 ppm. Fluvalinate residues in indoor-wintered colonies tended to be higher than in outdoor wintered colonies however these differences were not significant (Figure 3). The average fluvalinate concentration in wax following a winter-only treatment was 0.04 ppm. Fluvalinate residues in other studies have indicated that a recommended treatment result in the accumulation of .53 to .54 ppm (Slabezki et al. 1991; Nasar 2000). In Israel, extended treatments of six months have been shown to result in accumulations of 0.83 ppm (Slabezki et al. 1991). The accumulation of fluvalinate in the wax taken from brood chambers followin winter treatment was considerably less than that which would be expected for summer treatments.

In 1999-2000, groups of hives that received a fall treatment with Apistan were compared to those that received a fall and winter treatment.

Pretreatment and postreatment levels of varroa mites, tracheal mites and nosema for each beekeeper are shown in figures to . One beekeeper had moderate levels of varroa mites and low levels of tracheal mites while the other had moderate levels of tracheal mites and relatively low levels of varroa at the time initial samples were taken. Apistan treatments left in during fall and winter were more effective in controlling varroa than fall-only treatments. Levels of tracheal mites and nosema were not affected by the Apistan treatment.

Contact by bees with the Apistan strip during the fall and winter was found to vary considerably between hives. Contact of the honey bee cluster with the region of the hive where strips were located was monitored for a total of 189.7 days from October to April, 2000. Contact with the strip in colonies where Apistan was left in all winter ranged from 25.2 to 157.2 days (mean 83.8) in the outdoor-wintered colonies and from 6.3 to 156.6 days (mean 97.5 days) in the indoor-wintered colonies. Contact with the region of the hives where strips were located (including the period after they were removed in late fall) was similar to that found above for indoor wintered colonies 13.7 to 164.9 days (mean 98.0) but averaged only 25.99 days in colonies that received no winter Apistan treatment. The low contact values for the control group resulted from high levels of colony mortality.

The cluster size of colonies on 22 March was significantly greater in colonies that received a fall and winter Apistan treatment than in colonies that received only a fall treatment. This trend was statistically "similar" in both indoor and outdoor wintered colonies however the difference between treatments was much greater in outdoor wintered colonies than in indoor wintered colonies. Mean colony population size and colony brood area was also assessed on 27 April both colony population size and brood area were greater in colonies treated with Apistan

during fall and winter than in colonies treated only in the fall. These trends were similar in both of the beekeeping operations studied.

Fluvalinate residues appeared to vary considerably between treatment groups in 1999-2000. For example, the fluvalinate residue in a top box of a outdoor treated colony was 2.14 ppm. Whereas, the Fluvalinate residue from the bottom box of a outdoor wintered colony was 0.293 ppm.

Table 1: Mean abundance of varroa mites, mean prevalence of tracheal mites and mean density of nosema in honey bee colonies exposed to winter treatments with apistan compared to untreated colonies. Data show pretreatment levels of colonies that died over the winter and compare them to levels in colonies that survived. Post treatment varroa levels in surviving colonies are also reported.

	Wintered Indoors				Wintered Outdoors			
	Apistan[1]		No Apistan		Apistan		No Apistan	
	Dead n[2]=2	Live n=6	Dead n=2	Live n=6	Dead n=4	Live n=4	Dead n=6	Live n=2
Varroa[3] 21 Aug.	0.2	0.4	0.1	0.3	0.4	0.4	0.3	0
Varroa 9 Nov.	3.4	6.5	0.2	0.4	3.0	1.5	0.9	2.5
Varroa 17 May		1.8		2.7				3.5
Tracheal[4] 21 Aug.	4.5	0.5	0	1.5	2.6	1.5	1.3	0.5
Tracheal 9 Nov.	48.5	15.1	6	14.8	20.0	16	13.3	5.5
Nosema[5] 21 Aug.	7.5	0.7	1.0	0.5	2.8	9.0	0.7	3.0
Nosema 9 Nov.	1.0	1.0	0.0	3.0	0.0	0.0	2.1	1.0

1. Apistan treatments took place after the 9 November Sample
2. N= number of colonies living or dead
3. Mean number of mites per 100 bees
4. Mean percentage of bees with at least one mite.
5. Mean number of spores per bee x 1,000,000.

Figure 1: Pretreatment levels of *Varroa destructor* in colonies of honey bees sampled on the 9th of November 1989.

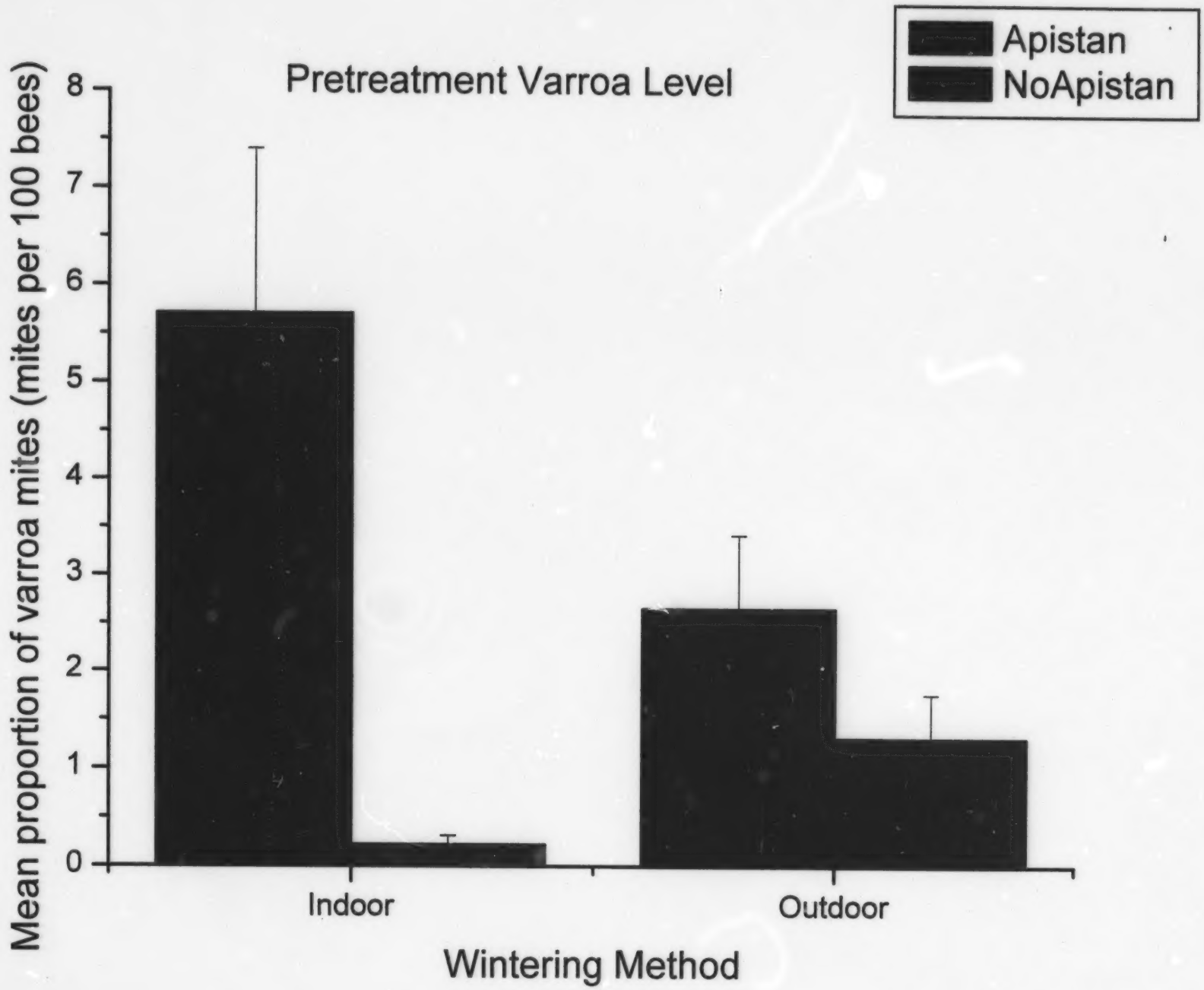

Pretreatment Varroa Level
Apistan
NoApistan
Mean proportion of varroa mites (mites per 100 bees)
0
1
2
3
4
5
6
7
8
Indoor
Outdoor
Wintering Method

Figure 2: Pretreatment levels of *Acarapis woodi* in colonies of honey bees sampled on the 9th of November 1989.

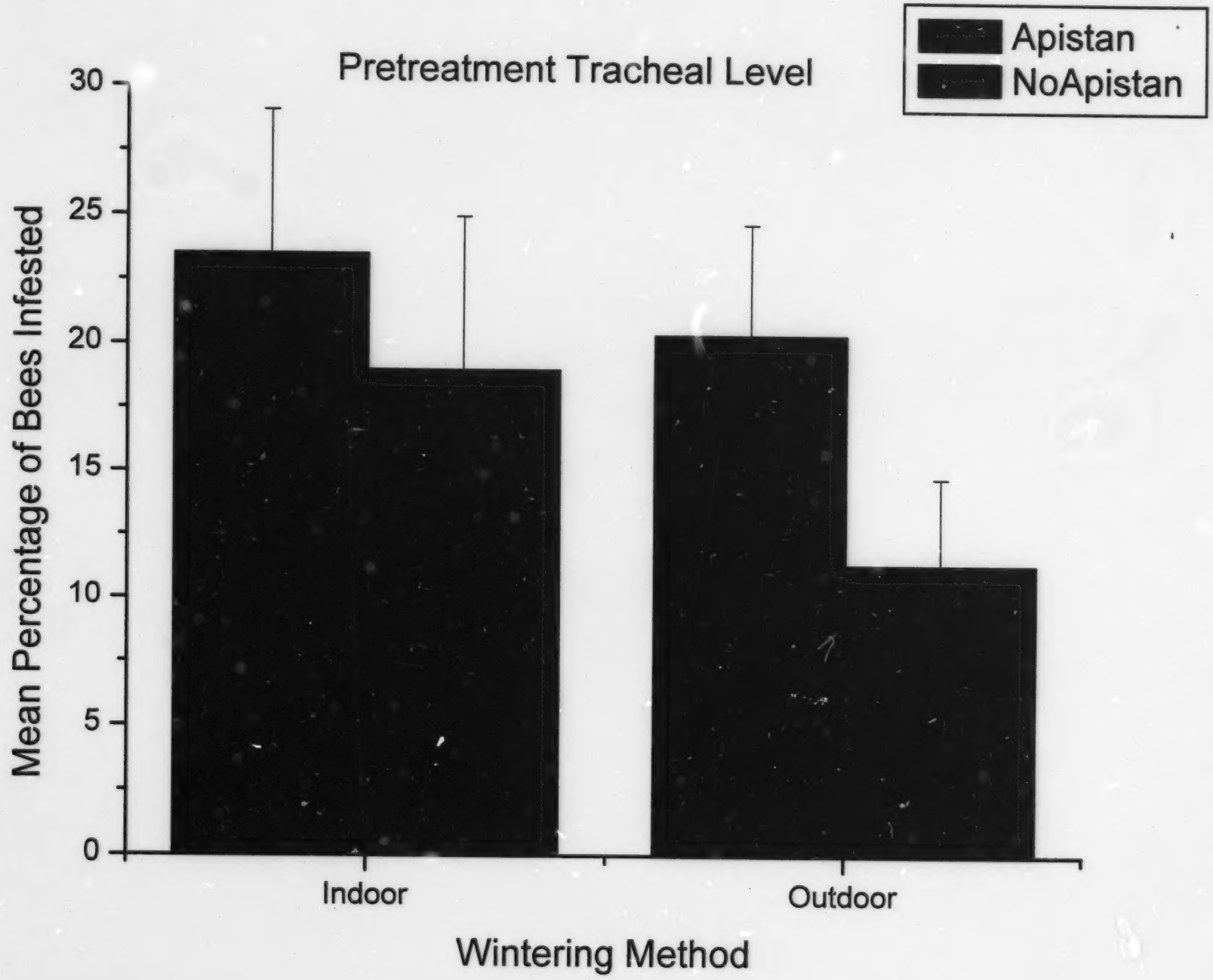

Pretreatment Tracheal Level
Apistan
NoApistan
Mean Percentage of Bees Infested
30
25
20
15
10
5
0
Indoor
Outdoor
Wintering Method

Figure 3: Pretreatment levels of *Nosema Apis* in colonies of honey bees sampled on the 9th of November 1989.

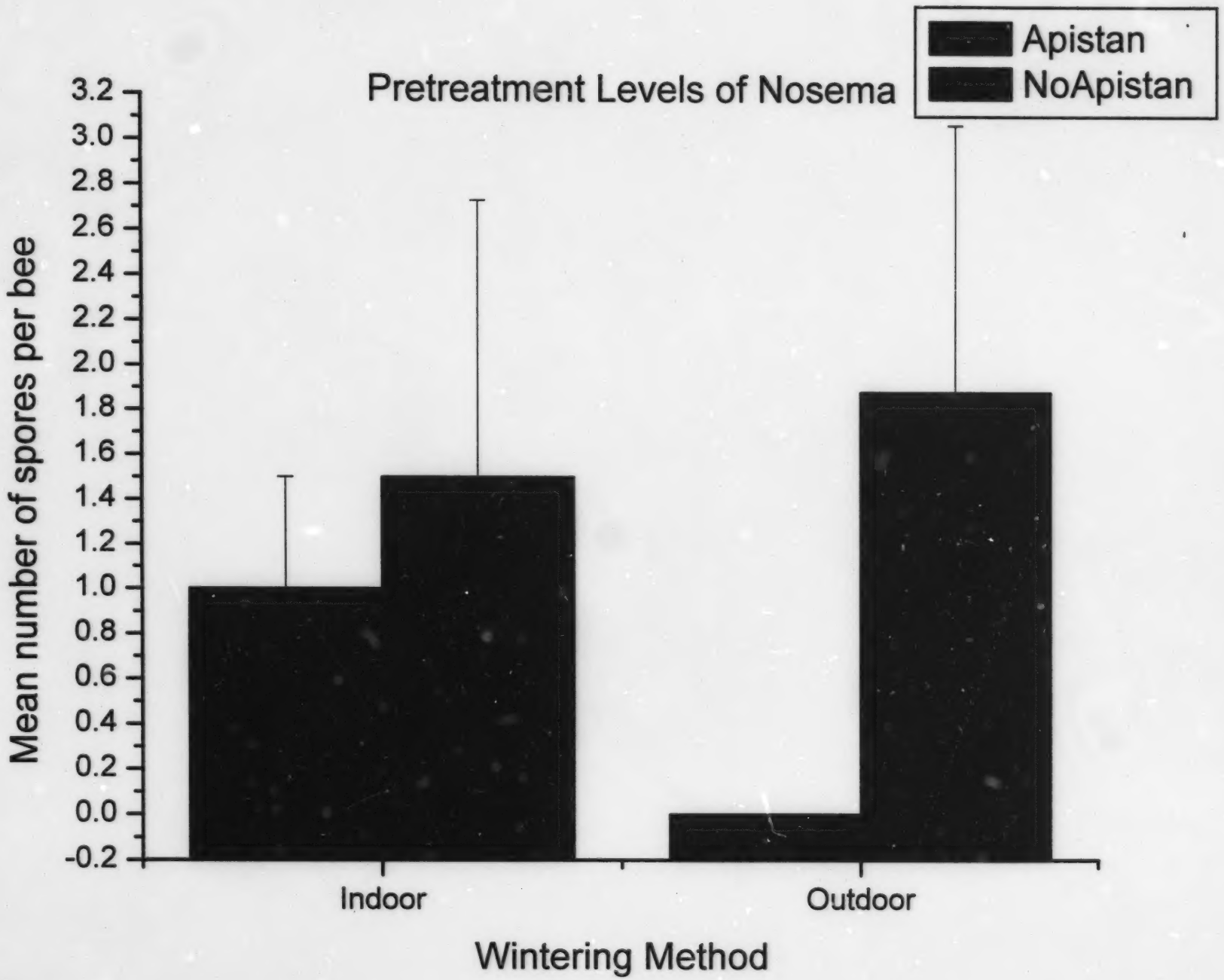

Pretreatment Levels of Nosema
Apistan
NoApistan
Mean number of spores per bee
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
Indoor
Outdoor
Wintering Method

Figure 4: Average spring worker population of honey bee colonies treated with Apistan during the winter and compared to untreated colonies in groups of colonies wintered indoors or outdoors (n=8). Colonies were sampled on the 29th April, 1999.

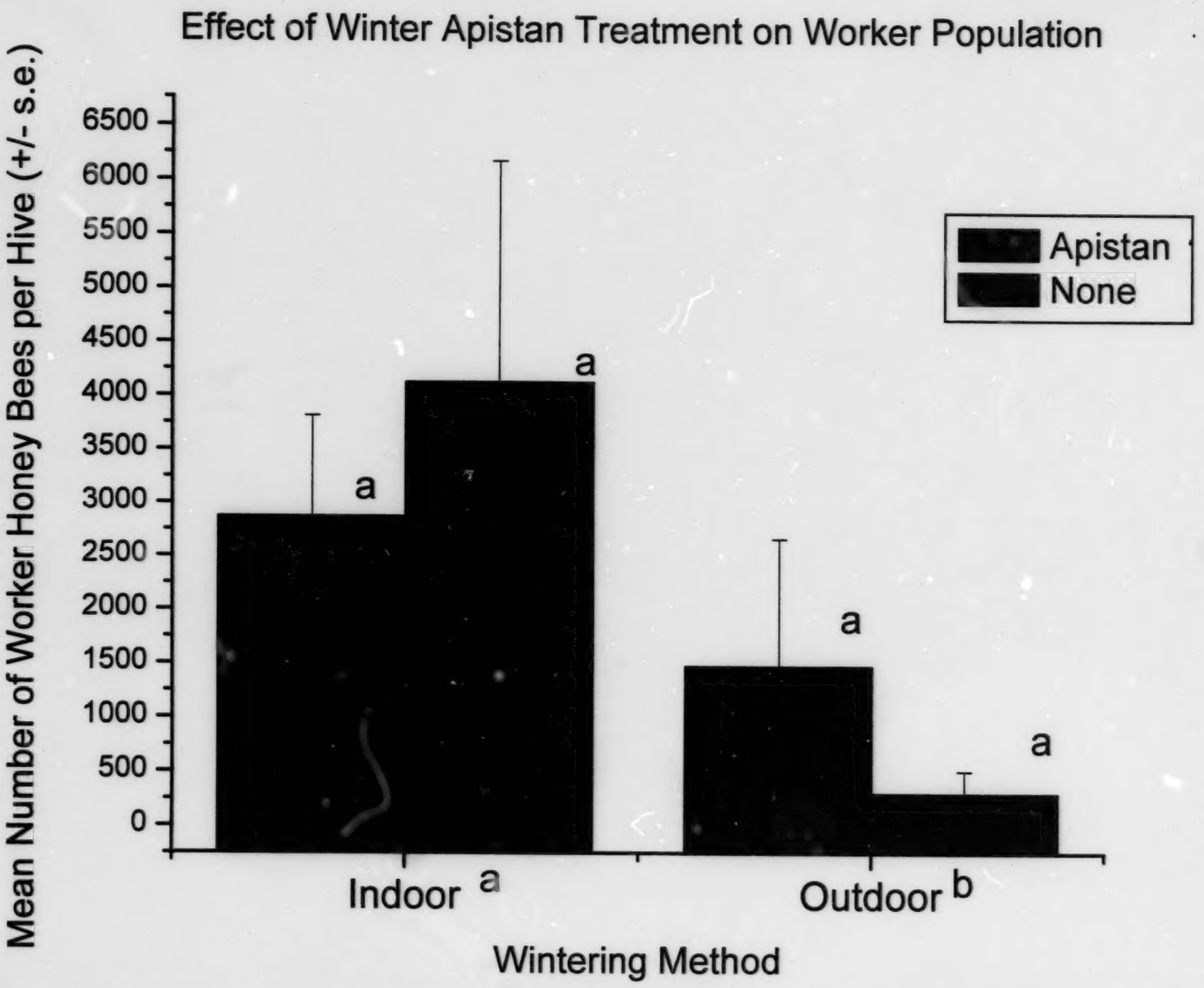

Effect of Winter Apistan Treatment on Worker Population
Mean Number of Worker Honey Bees per Hive (+/- s.e.)
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Apistan
None
a
a
a
a
Indoor a
Outdoor b
Wintering Method

Figure 5: Average worker brood population of honey bee colonies treated with Apistan during the winter and compared to untreated colonies in groups of colonies wintered indoors or outdoors (n=8). Colonies were sampled on the 29th April, 1999.

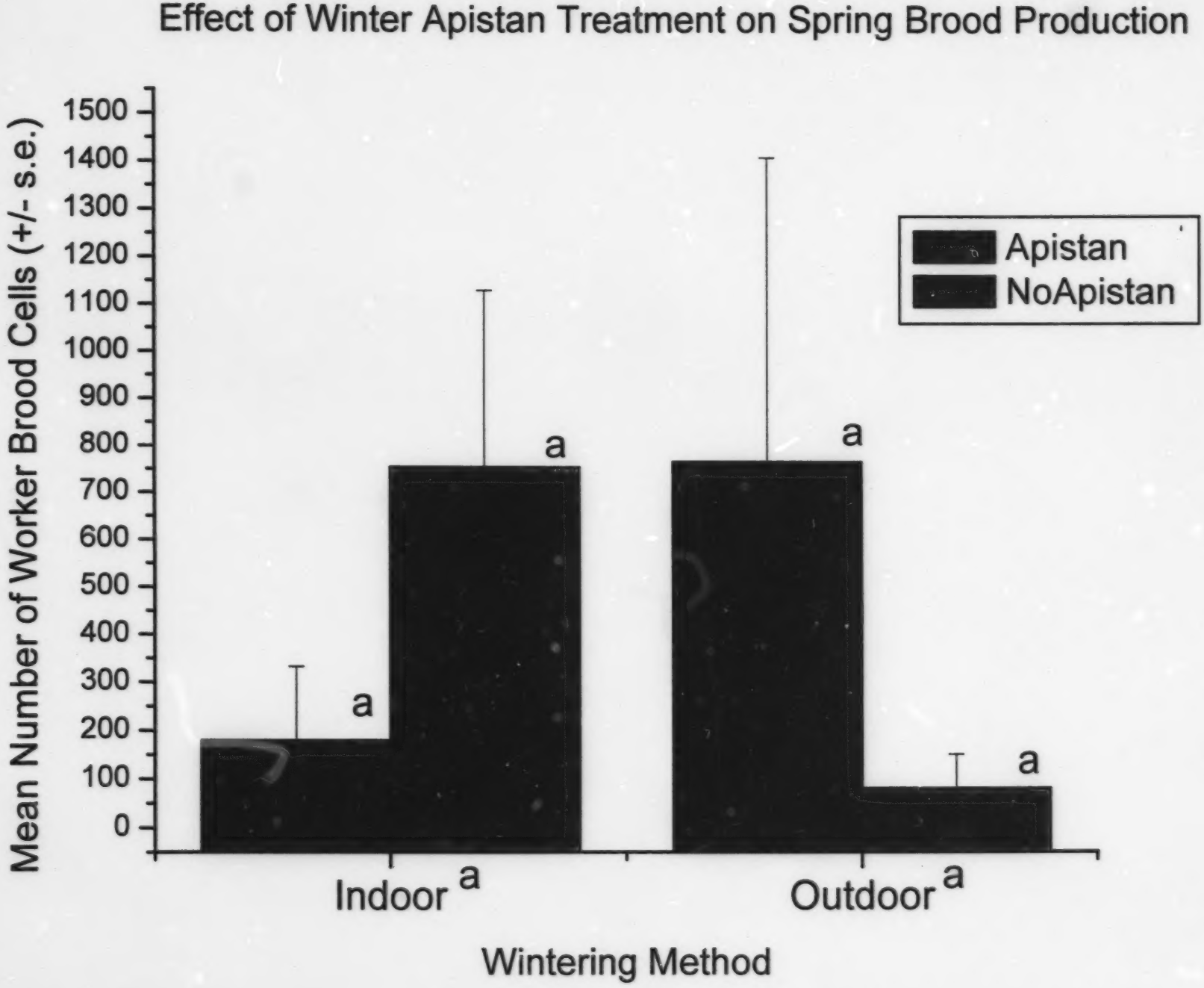

Effect of Winter Apistan Treatment on Spring Brood Production
Mean Number of Worker Brood Cells (+/- s.e.)
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
Apistan
NoApistan
a
a
a
a
Indoor
Outdoor
a
Wintering Method

Figure 6: Average fluvalinate concentration in wax comb taken from honey bee colonies treated with Apistan during the winter and compared to untreated colonies in groups of colonies wintered indoors or outdoors (n=8). Colonies were sampled on the 29th April, 1999.

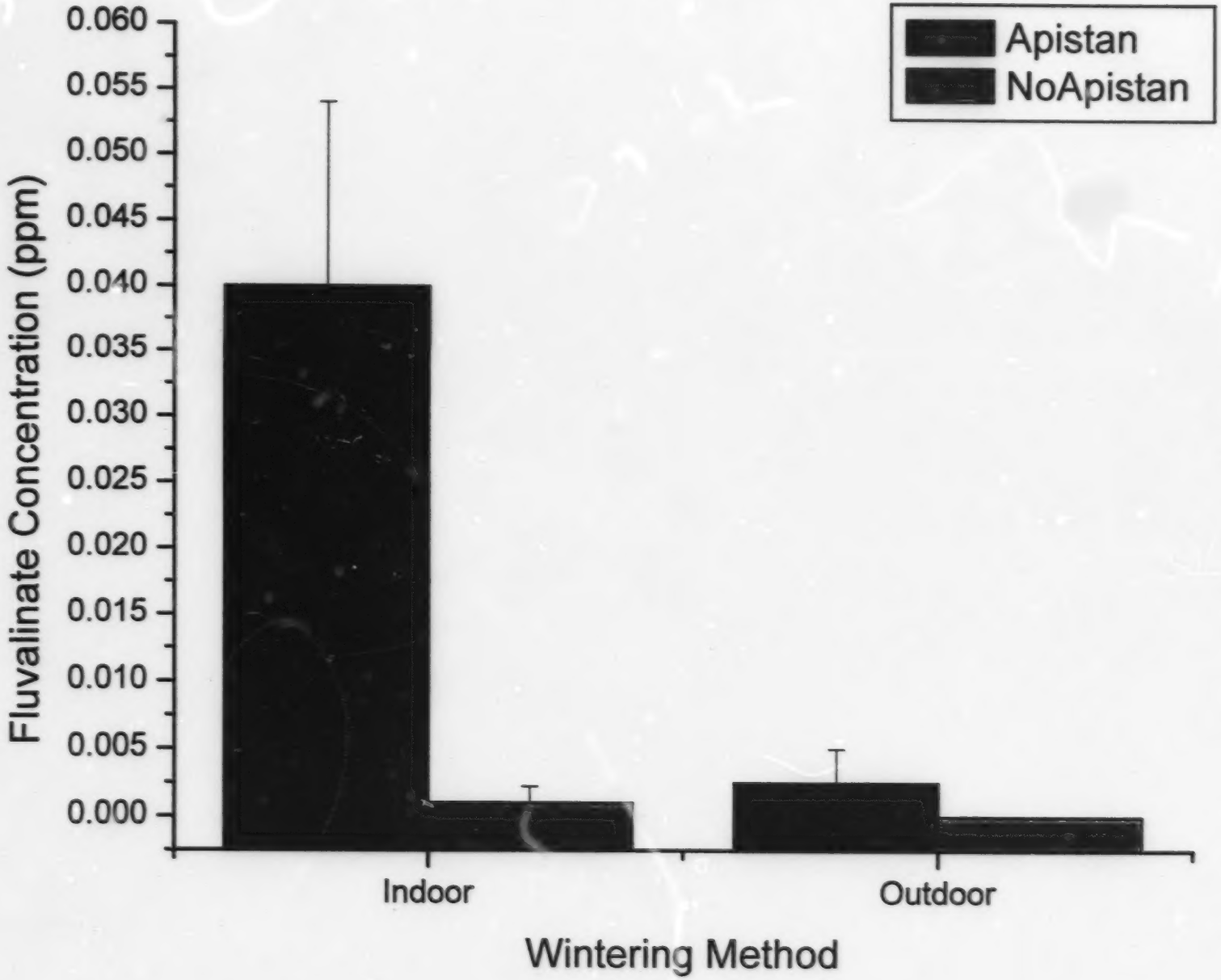

Apistan
NoApistan
Fluvalinate Concentration (ppm)
0.060
0.055
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
Indoor
Outdoor
Wintering Method

Figure 7: Pretreatment levels of *Varroa destructor*, *Acarapis woodi* and *Nosema Apis* in colonies from each producer that were used in the experiment in 1999/2000.

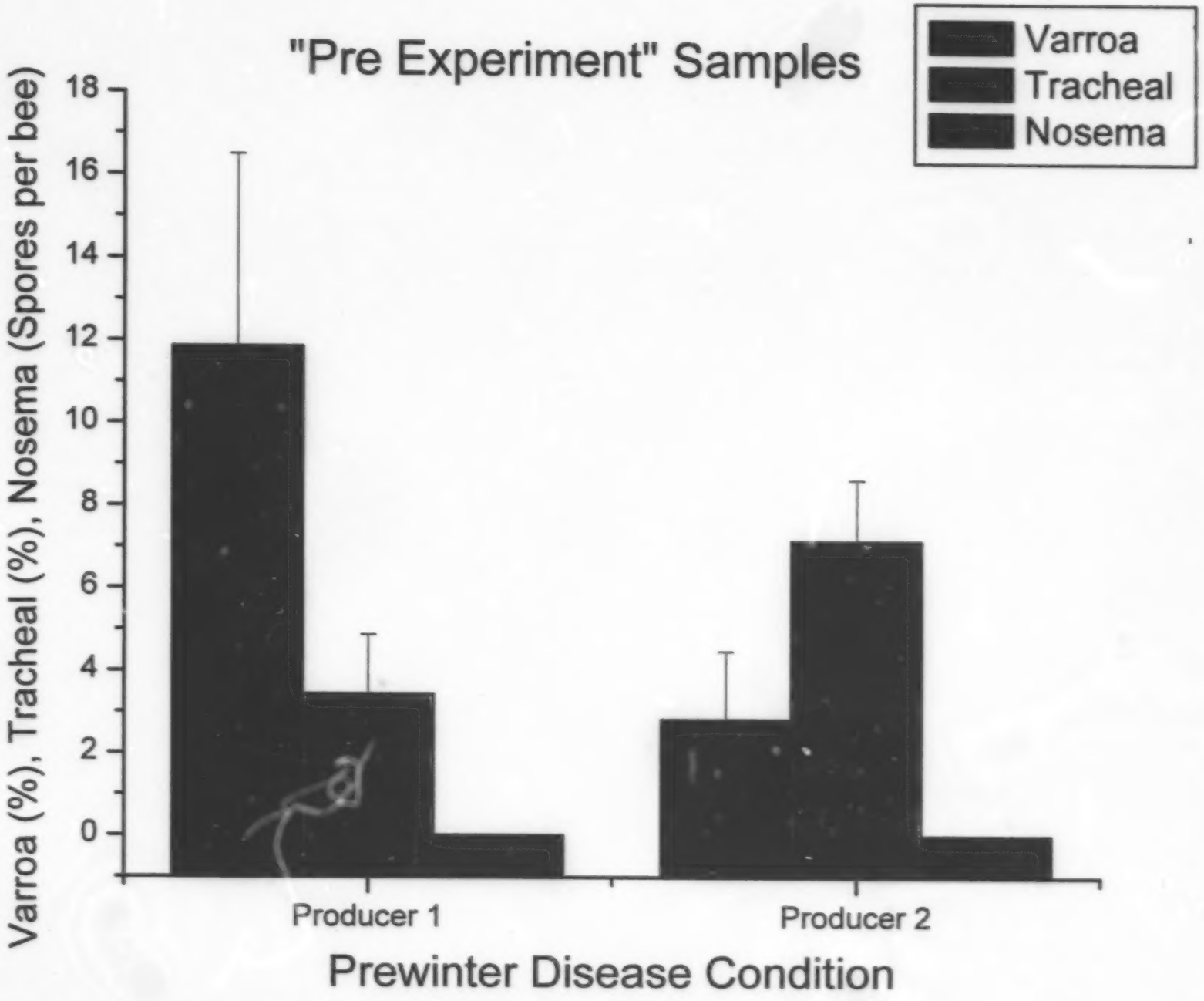

"Pre Experiment" Samples
Varroa
Tracheal
Nosema
Varroa (%), Tracheal (%), Nosema (Spores per bee)
18
16
14
12
10
8
6
4
2
0
Producer 1
Producer 2
Prewinter Disease Condition

Figure 8: Pretreatment levels of *Varroa destructor*, *Acarapis woodi* and *Nosema Apis* in colonies that were randomly assigned to fall only, and fall and winter treatments of apistan in 1999/2000 n=16.

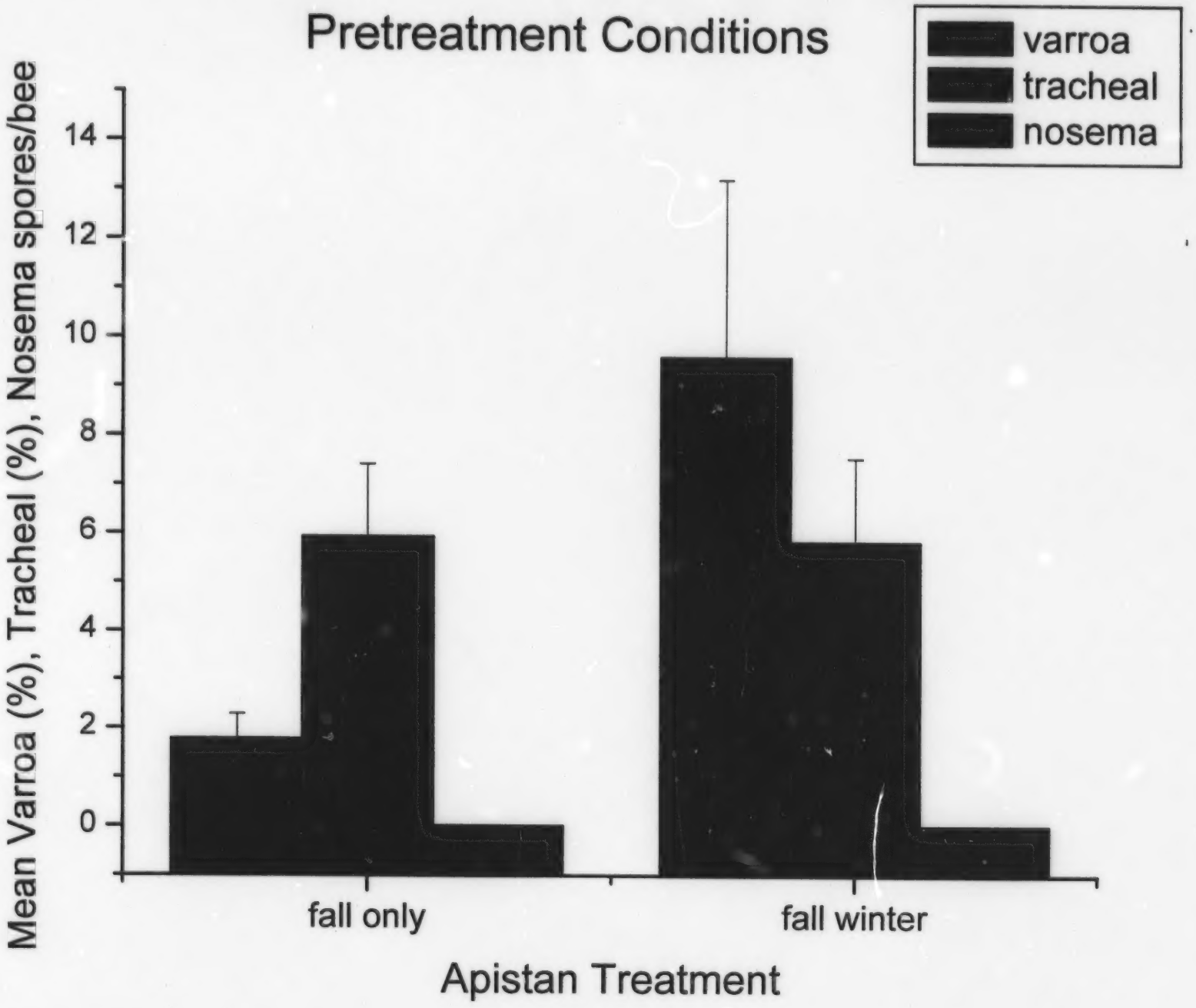

Pretreatment Conditions
varroa
tracheal
nosema
Mean Varroa (%), Tracheal (%), Nosema spores/bee
0
2
4
6
8
10
12
14
fall only
fall winter
Apistan Treatment

Figure 9: Effect of fall and winter treatment with apistan and fall only treatment with apistan on mean spring cluster rank of colonies wintered indoor and colonies wintered outdoors (n=8).

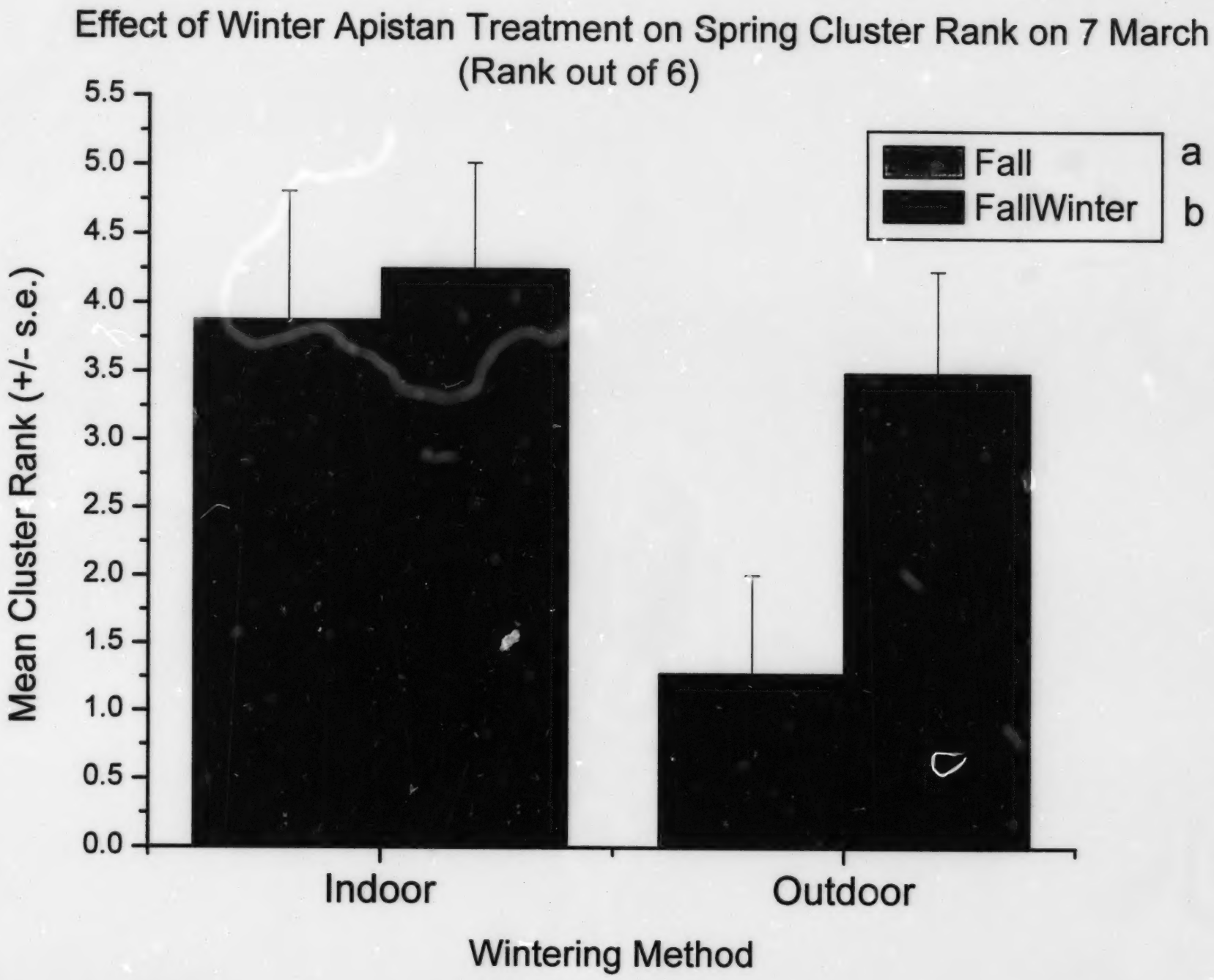

Effect of Winter Apistan Treatment on Spring Cluster Rank on 7 March
(Rank out of 6)
Mean Cluster Rank (+/- s.e.)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Fall
FallWinter
a
b
Indoor
Outdoor
Wintering Method

Figure 10: Effect of fall and winter treatment with apistan and fall only treatment with apistan on spring worker population size of colonies wintered indoor and colonies wintered outdoors (n=8).

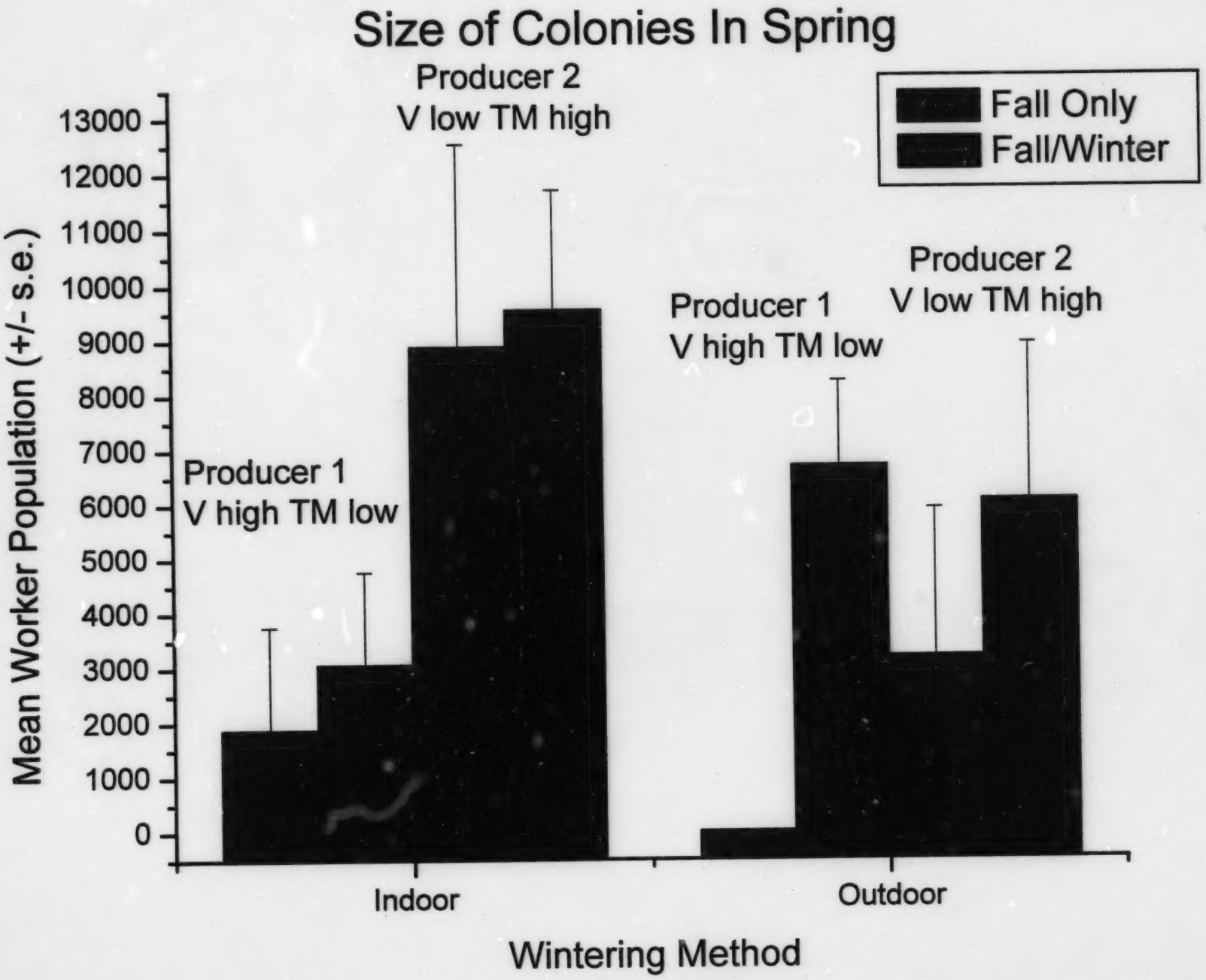

Size of Colonies In Spring
Producer 2
V low TM high
Fall Only
Fall/Winter
Producer 1
V high TM low
Producer 2
V low TM high
Producer 1
V high TM low
Mean Worker Population (+/- s.e.)
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Indoor
Outdoor
Wintering Method

Figure 11: Effect of fall and winter treatment with apistan and fall only treatment with apistan on spring brood area (square cm of broo) of colonies wintered indoor and colonies wintered outdoors (n=8).

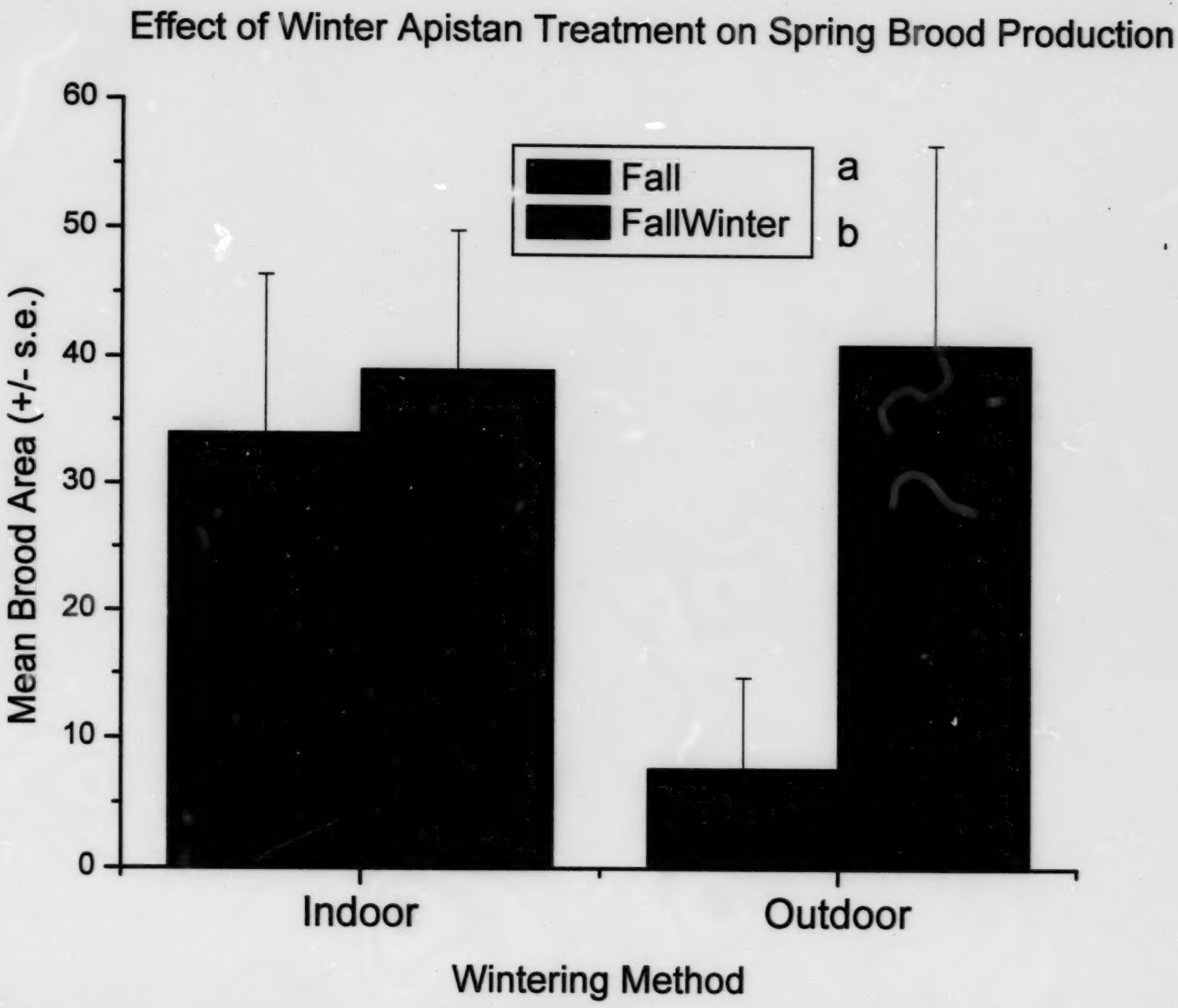

Effect of Winter Apistan Treatment on Spring Brood Production
Mean Brood Area (+/- s.e.)
60
50
40
30
20
10
0
Fall
FallWinter
a
b
Indoor
Outdoor
Wintering Method

Figure 12: Effect of fall and winter treatment with apistan and fall only treatment with apistan on varroa mite level of colonies wintered indoor and colonies wintered outdoors (n=8). Black bars represent fall samples and grey bars indicate samples collected in spring.

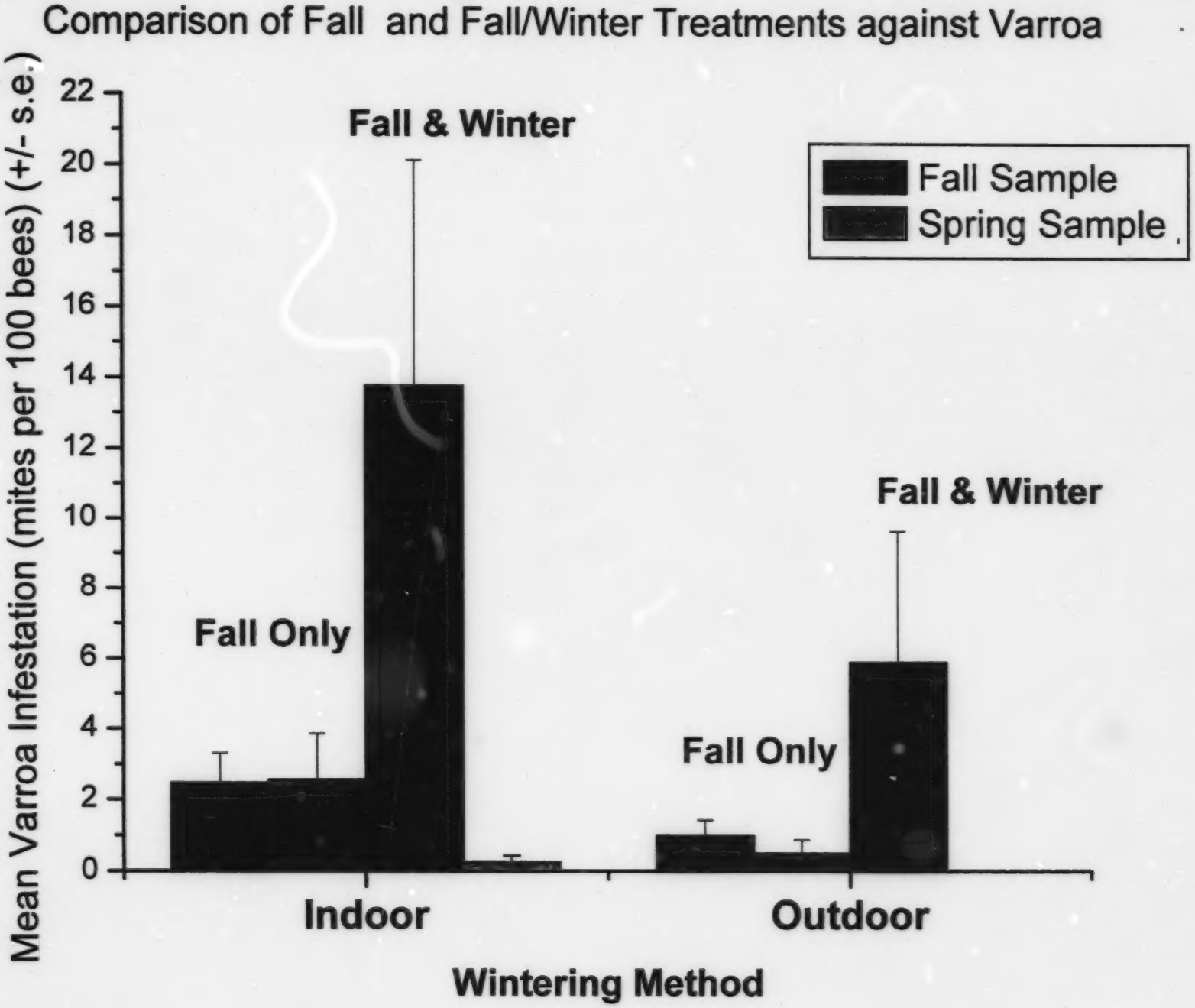

Comparison of Fall and Fall/Winter Treatments against Varroa
Mean Varroa Infestation (mites per 100 bees) (+/- s.e.)
22
20
18
16
14
12
10
8
6
4
2
0
Fall & Winter
Fall Sample
Spring Sample
Fall Only
Fall & Winter
Fall Only
Indoor
Outdoor
Wintering Method

Figure 13:Effect of fall and winter treatment with apistan and fall only treatment with apistan on tracheal mite level of colonies wintered indoor and colonies wintered outdoors (n=8). Black bars represent fall samples and grey bars indicate samples collected in spring.

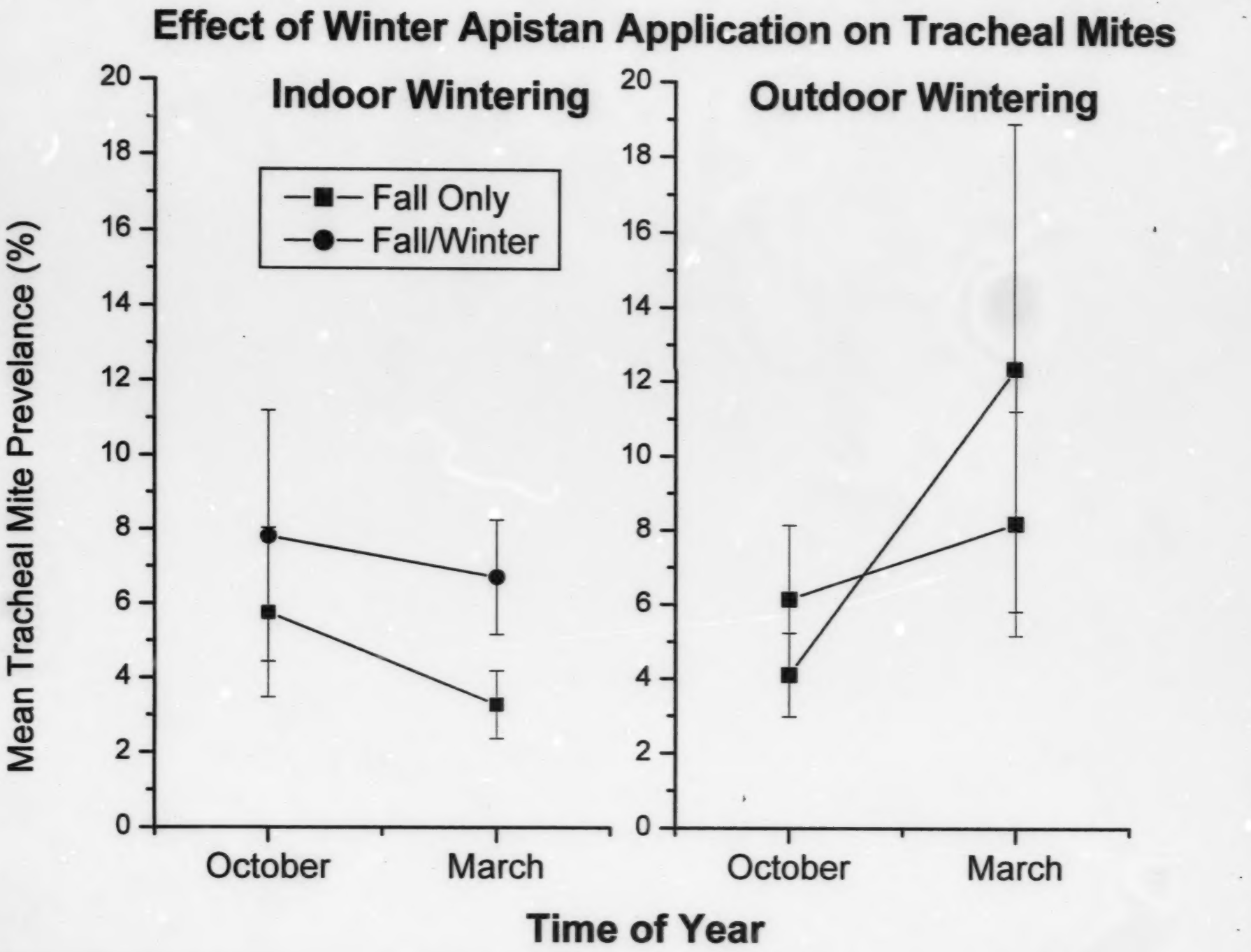
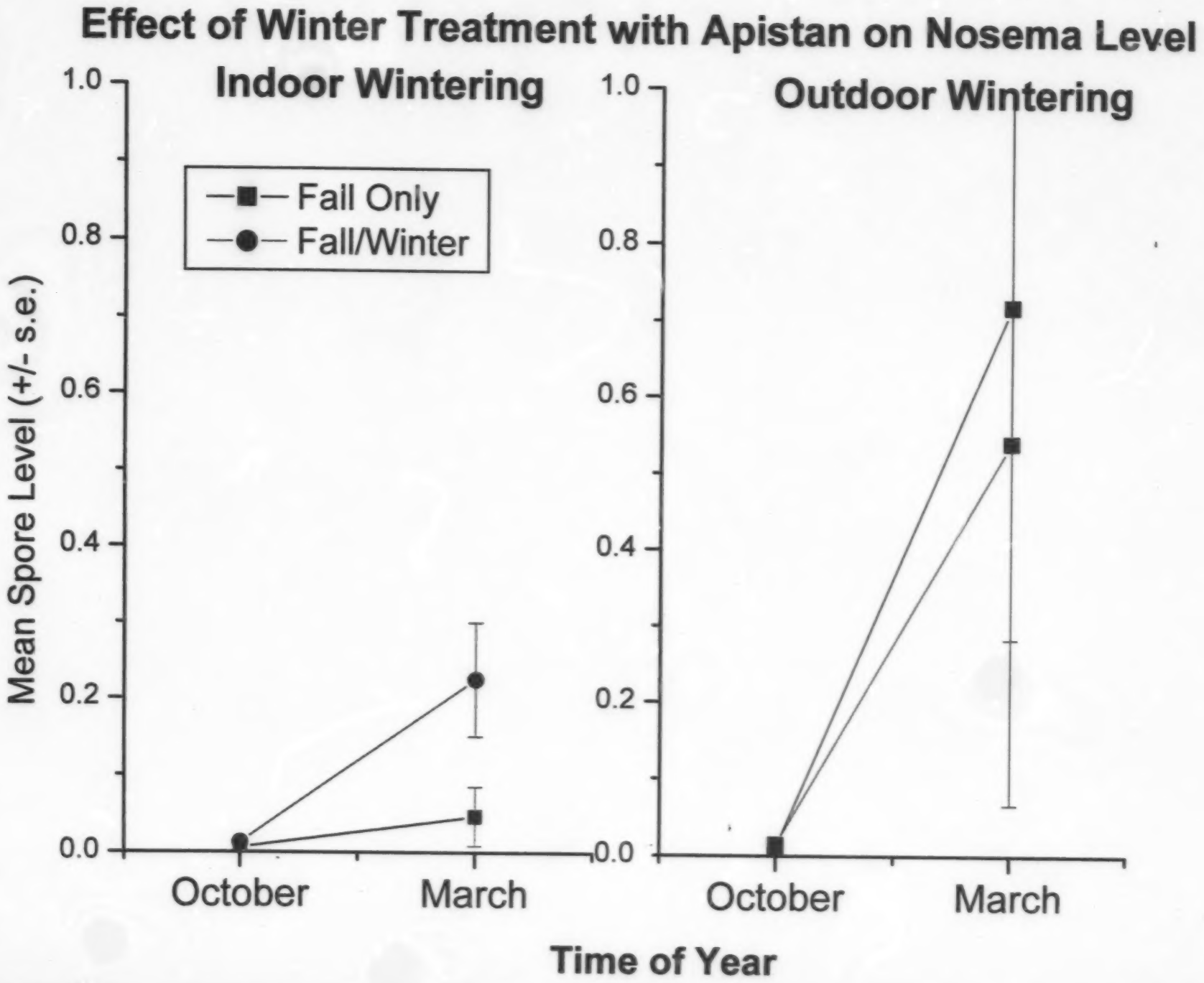

Effect of Winter Apistan Application on Tracheal Mites
Indoor Wintering
Outdoor Wintering
Fall Only
Fall/Winter
Mean Tracheal Mite Prevelance (%)
0
2
4
6
8
10
12
14
16
18
20
October
March
Time of Year

Figure 14:Effect of fall and winter treatment with apistan and fall only treatment with apistan on nosema infestation level of colonies wintered indoor and colonies wintered outdoors (n=8). Black bars represent fall samples and grey bars indicate samples collected in spring.


Effect of Winter Treatment with Apistan on Nosema Level
Indoor Wintering
Outdoor Wintering
Fall Only
Fall/Winter
Mean Spore Level (+/- s.e.)
1.0
0.8
0.6
0.4
0.2
0.0
October
March
Time of Year

Discussion

Winter Apistan treatments did not have a negative impact on colony survival or spring brood production either in colonies that were treated in winter only and compared to those left untreated or in those colonies that were treated in both fall and winter and compared to those treated only in the fall. Our two year study showed that when both tracheal and varroa mites were present, the practice of leaving Apistan strips in colonies resulted in a trend towards larger colonies and greater colony survival in one year and significantly larger colonies and a trend towards greater colony survival in the second year. This was the case whether colonies had more varroa than tracheal mites or visa versa. When both species of mites are present it appears that honey bee worker survival and, in turn, colony survival can be dramatically increased by reducing the winter population of varroa mites to near zero. The risk of any negative impacts would seem to be minimal as the effects of leaving strips in colonies throughout the winter in the absence of varroa mites also does not negatively effect colony size or brood production (Westcott and Winston).

In both years colony survival and population size was higher in colonies wintered indoors than in colonies wintered outdoors. The difference between treatments was also smaller in indooor wintered colonies than in outdoor wintered colonies. This would seem to indicate that indoor wintered colonies were more tolerant of higher mite infestation levels than were colonies wintered outdoors. Indoor wintering seemed to favour the populations of varroa mites whereas outdoor wintered colonies seemed to favour the development of tracheal mite populations.

While the practice of leaving Apistan strips in colonies throughout the winter seems to be clearly advantages when both tracheal and varroa mites are present the effects on the possible development of aracide resistance must also be considered. The label recommendation for Apistan use in Canada states that the strips must not be left in colonies for more than 42 days. The concern in leaving strips in for periods of longer than 42 days relates first to the fact that selection would occur against a larger number of generations of mites thus enhancing resistance and second to the potential for increased residue buildup in the comb. Our results suggest that contact with the strip is not constant throughout the winter. Bees had contact with the strip for approximately half of the 189 day monitoring period. This clearly exceeds the recommended time on the label, however, in the first year of our study the amount of residue built up in the comb was less than that one would expect to see from a normal spring application. Thus, in the Canadian prairie region the practice of treating in the fall and leaving the strips in all winter would be expected to result in less residue than if a beekeeper had to treat in both fall and spring.

During the winter period on the Canadian Prairies there is very little brood rearing. Thus mite reproduction would be greatly reduced or absent. Therefore the longer exposure period would not result in selection over a larger number of generations. Therefore we would predict that under the type of environmental conditions found on the Canadian prairies that leaving strips in throughout the winter period should not speed up the rate of development of acaricide resistance in varroa mites.

Project Activities:achievments papers written

Papers based upon this research were presented at the following meetings. A manuscript based upon the research will be submitted to a refereed scientific journal.

Currie, R.W. 2001. Winter Treatments With Apistan. Manitoba Beekeepers Association 91th Annual Meeting. Winnipeg, Manitoba. February.

Currie, R.W. 2001. Winter Treatments With Apistan. Annual Meeting of the Saskatchewan Beekeepers Association. Saskatoon, Saskatchewan. February.

Indications for Further Development

The results of this study showed that leaving Apistan strips in colonies throughout the winter did not negatively impact colony development. The ability to leave Apistan strips in colonies would reduce producer's input costs by eliminating the need to crack colonies open to remove the strips. We also showed that there is a substantial economic benefit associated with leaving strips in colonies when both the varroa and tracheal mite are present in colonies. The amount of time spent in contact with the strip using this application method exceeds that found on the current label recommendation. Further analysis of comb residues will give important information on the potential impact of this practice on the development of pesticide resistance in mites. This data should be carefully considered in determining wether a change in the pesticide label recommendation is warranted.

Personnel

This project was carried out by: the principle researcher Dr. R. W. Currie, University of Manitoba; collaborator Dr. Desiree Vanderwel, technicians Lisa Babey and Linda Klymochko and the following undergraduate and graduate students: Nicole Froese, Deborah Wytrykush, Kristina Pohl, Monica Pazerniuk, Robyn Underwood and Nicole Lauro.

Equipment

No major equipment was purchased to conduct this research.

Project Photos:

Photos of Dr. Desiree Vanderwel and Monica Prazerniuk conducting analysis of wax samples are contained on the enclosed disk.

Innovation Fund Acknowledgment

The Saskatchewan Agri-Food Innovation Fund has been acknowledged in all oral and written presentations made on this research. It will also be acknowledged in all resulting scientific

publications.

Expense Statement

Final expense statements have been previously submitted.

Literature Cited

Frison, S., Breitkreitz, Currie, R. Nelson, D and P. Sporns. (1999) The analysis of fluvalinate in beeswax using GC/MS. Food Research International. 32:35-41

Nasar, M. 2000. Miticide residues in honey and beeswax from Ontario. The Sting 18(6):32-35

Slabezki, Y, H. Gal and Y. Lensky. 1991. The effect of fluvalinate application in bee colonies on population levels of Varroa jacobsoni and honey bees (Apis mellifera L.) and on residues in honey and wax. Bee Science. 1(4):189-195.